**Short Communication**

# Effect of inclusion or exclusion of epithelial cells in equine respiratory cytology analysis


## Abstract

There is variation amongst published studies as to whether epithelial cells are included in differential counts for tracheal wash (TW) and bronchoalveolar lavage (BAL) cytology in horses. The aim of this study was to determine whether inclusion / exclusion of epithelial cells affects interpretation of airway cytology. Using criteria of >20% TW neutrophils, >10% BAL neutrophils and >5% BAL mast cells to indicate airway inflammation, 21%, 4% and 8% of horses changed from being categorised as 'normal' to 'abnormal' when epithelial cells were excluded from differential counts. It is recommended that future equine respiratory research studies explicitly state whether epithelial cells are included or excluded in differential counts. A consensus on epithelial cell inclusion during cytology reporting is required.



Keywords: horse, tracheal wash, bronchoalveolar lavage, cytology, epithelial cell

There is variation amongst published studies as to whether epithelial cells are included in differential counts for tracheal wash (TW) and bronchoalveolar lavage (BAL) cytology. A published review of respiratory cytology from poorly performing horses revealed 4 of 6 studies included epithelial cells in differential counts for TW and 7 of 13 for BAL (Richard et al. 2010). Utilising this published data, the mean proportion of epithelial cells was 33% for the TW studies and 5% for BAL studies. In many studies specific 'cut offs' for the proportion of neutrophils, mast cells and eosinophils have been used to indicate abnormal airway inflammation. However, these recommendations have been determined from studies and subsequently applied to studies when epithelial cells are both included and excluded in differential counts (Couetil et al. 2016, Christley et al. 2001, Hodgson 2002, Malikides et al. 2003, da Silva et al. 2017, Sweeney et al. 1992). In other studies, it is unclear whether differential counts were obtained from total nucleated cells or total inflammatory cells. Knowledge of epithelial cell proportions may be of clinical value. In the UK, where TW are widely performed as part of routine clinical racehorse practice, differentials including epithelial cells are used in some clinics to provide recommendations for suitability to race. Further research is required to improve our understanding of the circumstances which may affect epithelial cell proportions; an increase in BAL epithelial cells is reported in horses after exercising in cold air (Davis et al 2002) and they have been used as markers of mucosal injury in humans and laboratory animals (Davis et al 2002).

The aim of this study was to determine whether inclusion or exclusion of epithelial cells affects the interpretation of airway cytology in TW and BAL. A convenience sample of TW (n=100) and BAL (n=50) cytology findings from poorly performing horses over a two-year period were reviewed retrospectively. The TW study population comprised 89 Thoroughbreds and 11 mixed breeds; 22 mares, 88 geldings; with a mean age of 6 years (2-14years). The BAL study population comprised 44 (88%) Thoroughbreds and 6 (12%) mixed breeds; 9 mares, 41 geldings; with a mean age of 6 years (2-15 years). Tracheal wash samples were obtained transendoscopically using 20ml sterile saline. The BAL samples were obtained from one lung using 300ml sterile saline in a single bolus. Differentials had been obtained by board certified pathologists from Cytospin preparations stained with modified Wright's stain. Slides were examined to assess cellularity, types and morphology before performing a manual differential count of 200 cells. Differential counts were obtained with respiratory epithelial cells included

and were later recalculated to determine proportions with respiratory epithelial cells excluded. If cells were present in sheets or large clumps, these were not included, because the uneven distribution of cells would have the potential to skew the differential count. For the tracheal wash >20% neutrophils were used to indicate airway inflammation (Hodgson 2002). Earlier guidelines suggested that >5% neutrophils, >2% mast cells and >1% eosinophils in BAL indicated airway inflammation. However, the most recent consensus statement advised that >10% neutrophils, >5% mast cells and >5% eosinophils, regardless of BAL technique indicated airway inflammation (Couetil et al. 2016), with values in-between likely equivocal and technique dependent. Therefore, for the purposes of this study BAL neutrophils >10% neutrophils, mast cells >5% and eosinophils >5% eosinophils were used to indicate airway inflammation.

The median proportion of epithelial cells in the TW was 37% (Interquartile range (IQR) 15-74%). When epithelial cells were included in differential counts 41% of horses had greater than 20% neutrophils. When epithelial cells were excluded from the differential count a further 21% of horses had greater than 20% neutrophils, resulting in 62% of horses being categorised as abnormal (table 1).

The median proportion of epithelial cells in BAL was 6% (IQR 2-10%). When epithelial cells were included 24% of horses had >10% neutrophils. A further 4% of horses changed from being categorised as normal to being categorised as abnormal when epithelial cells were excluded. When epithelial cells were included 6% had >5% mast cells; an additional 8% of horses became categorised as abnormal when epithelial cells were excluded (table 1). No horse had >5% eosinophils.

The purpose of this study was to highlight the extent to which inclusion/exclusion of epithelial cells might affect interpretation of airway cytology. There was a higher proportion of epithelial cells in TW samples than BAL samples, which has the potential to more greatly influence interpretation. Determination of epithelial cell proportions from TW may be warranted as they represent a large proportion of the normal cells. The smaller epithelial cell proportions in BAL, meant that exclusion of epithelial cells resulted in a change of category in a smaller number of horses than the TW.

Variation is apparent in the reporting of cytological findings from sputum and BAL samples in humans. In sputum cytological analysis, there is a lower proportion of respiratory epithelial cells (~1.5%) (Spanevello et al 2000) than in equine TW samples. Whereas the proportion of epithelial cells in human and equine BAL samples is similar (Meyer et al 2012). For sputum analysis squamous epithelial cells are excluded but bronchial epithelial cells are included in differential cell counts (Weiszhar and Horvath 2013). Whereas guidelines for human BAL reporting advise the use of differential counts for immune/inflammatory cells only (Meyer et al 2012). However, it is recommended that cytology reports do separately include the figure for epithelial cells as a percentage of total nucleated cells (Meyer et al 2012).

For equine respiratory cytology further studies are required to better understand the clinical value of reporting epithelial cells for both TW and BAL. Furthermore, the repeatability of epithelial cell counts warrants study, along with a consensus on how groups or sheets of epithelial cells or inflammatory cells should be considered. Although their presence can provide interpretative information, they were not included in the count because the uneven distribution was likely to skew the differential. It has been shown that the differential counts of less common cell types such as mast cells may be unreliable even at counts of 400 cells and that evaluation of 5 microscopic fields at 500x magnification had improved reliability (Fernandez et al 2013). The cell counts of samples included in this study were lower and the recalculation without epithelial cells means that the inflammatory cells counted were further reduced.

It is recommended that future equine respiratory research studies explicitly state whether epithelial cells are included or excluded in differential counts. A consensus on epithelial cell inclusion during cytology reporting is required.

| | Differential of total nucleated cells (epithelial cells included) | Differential of inflammatory cells (epithelial cells excluded) |
|---|---|---|
| Tracheal wash – Neutrophil proportion > 20% | 41% | 62% |
| BAL – Neutrophil proportion > 10% | 24% | 28% |
| BAL – Mast cell proportion > 5% | 6% | 14% |

Table 1: Shows the proportion of horses categorised with airway inflammation when epithelial cells were included and excluded in differential counts.

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